Review Article

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Haemoglobinopathies in Greece: prevention programme over the past 35 years

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At present, prevention of thalassaemia and sickle cell disease is the only realistic approach to control the birth of new patients in countries having high numbers of carriers. This is fully justified because avoiding the birth of an ever increasing number of patients may allow a more effective use of the available resources in improving the management of the patients surviving today and alleviate the already overloaded public health system from the inevitable tremendous and ever increasing cost. Moreover, prenatal diagnosis may help couples at risk to have non-thalassaemic children. Greece is one of the countries where the mean frequency of carriers is approximately 7.5 per cent (population 11 million) and has set up a nationwide programme for carrier identification in the early seventies; this is provided through a dozen of specific Units attached to the major Blood Transfusion Services of the country, on a voluntary basis and free of charge. Spread of information through mass media, the schools, and other groups has greatly contributed in creating the necessary sensitization; obstetricians and antenatal Clinics are also instrumental to this effect. Prenatal diagnosis is offered centrally (Athens) and covers satisfactorily the estimated needs (500-600 annually); the total number has already exceeded 35,000. According to information obtained from the major paediatric hospitals all over the country, the number of thalassaemia major or SCD admitted for treatment over the last ten years has been around 15 yearly (instead of an estimate of 120-130).

Key words Inherited disorders of haemoglobin - haemoglobinopathies - prenatal diagnosis - prevention - red cells - thalassaemia

The inherited haemoglobin disorders do not only cause suffering and unhappiness to the patients but also absorb a large part of resources and human effort in several countries which harbour the deleterious genes[1,2]. Greece, a country of approximately 11 million people belongs to this group; with a mean frequency of thalassaemia carriers at 7 per cent plus 1 per cent of carriers of haemoglobin S, and number of births surpassing 100,000 yearly, the total number of newborns carrying two deleterious genes, if no prevention measures are taken, is estimated to be about 120-130 per year.

Although accurate data are not available, it appears that this was the situation before and during the first decades after World War 2, but was somehow compensated as inadequate or no treatment was leading to the early death of the affected newborns. The problem started being pressing in the fifties and sixties, when infant mortality mainly for infections or malnourishment was drastically reduced, while transfusions and improvements in health care allowed thalassaemic infants to survive a miserable short life. From then on, thalassaemia and sickle cell disease started becoming a major problem not only for the

patients and their families, but also for the Public Health System which had to take care of the ever increasing cost of regular transfusions, iron chelation, frequent hospitalisations and general medical follow up. As a result, the total number of patients surviving today is estimated to be 4,000 thalassaemics plus a few hundreds of patients with homozygous HbS or compound β^S/β-thalassaemia[3,4].

Under present conditions, the cost for providing an acceptable, even if suboptimal, treatment to a thalassaemic child is estimated to be 20,000 US dollars yearly. This comprises mainly the cost of processing the blood and the cost of using chelating agents, and most probably will increase as a result of the introduction of newer agents in the market. Adding the expenses for running the transfusion service, the salaries of the medical and mainly paramedical staff, the cost of treating the inevitable complications (bile stones, leg ulcers, enlarged spleen, cardiomyopathy and cardiac failure, endocrinopathies and bone diseases, *etc*.) brings the expense to numbers which are difficult if not impossible, to meet especially considering the large and ever increasing number of patients[5].

Thalassaemia is a genetic disease, although the parents of affected children do not appear different than other persons who have healthy children, their red cells are smaller than normal and display an osmotic fragility also clearly lower than normal[6]; in contrast, their haemoglobin A_2 is significantly higher than normal. These criteria of heterozygous thalassaemia, repeatedly confirmed in all family studies of children with thalassaemia, opened the way towards larger population studies, which established the frequency and the distribution of the "trait " across the country. In parallel, the sickling test and haemoglobin electrophoresis defined the dispersion of the sickle cell gene. It was thus established that the mean frequency of heterozygous thalassaemia all over the country was 7.4 per cent[7,8] and that the distribution was extremely uneven, the low altitude fertile areas of Thessaly, Western Peloponnesus, and Western Epirus displaying frequencies up to 15 per cent, in contrast to high altitude areas and Macedonia, where the percentage of heterozygotes was significantly lower. The distribution of HbS showed more or less the same pattern, with specific concentrations in Orchomenos (Boetia; central Greece), western Peloponnesos, and the Chalkidiki peninsula, where the frequency of heterozygotes in some villages reached up to 20 per cent[4].

With these high numbers of patients, their dreadful appearance because of the facial deformities and the protuberant abdomen, the continuous pleas for blood to provide some more years of a miserable life, and the burden imposed on their families, it is not surprising that parents, relatives and the society eventually started demanding to know the measures to prevent this sad situation, that is they wanted to know whether they were carriers of the deleterious genes or not. This information was initially provided within the context of various clinico-laboratory studies or epidemiological surveys by the clinics where the sick children were being transfused or hospitalized, but this was clearly inadequate and, in most cases "retrospective". In other words, it was provided after a thalassaemic child was born. Gradually scientists started supporting the concept that carrier identification should be "prospective", *i.e.* should be provided prior to childbirth to all potential parents. Of the many names, Drs Phaedon Fessas and Spyros Doxiadis deserve special recognition for their contribution towards this[9].

At the same time, the idea of prospective carrier identification was also being implemented in other Mediterranean places, such as Sardinia[10], Ferrara, Torino and Milano (which hosted large numbers of migrants from Southern Italy), and Cyprus[11], where the frequency of haemoglobin disorders was high enough to justify massive screening, A WHO sponsored workshop in Geneva formulated the basic rules for carrier identification at large and prompted all scientists and authorities in countries with high incidence of these deleterious genes to implement this activity[12,13]. Of the many contributors in this process, the perseverance of Bernadette Modell in the UK "to stamp out thalassemia", the systematic approach of screening of Antonio Cao in Sardinia and the administrative efficiency of Anver Kuliev, at that time in Geneva/WHO, deserve special mentioning[12,13].

Selecting the right target group for the prospective screening tests was the first major issue to address; blood testing in high school children was an option, but it did not appear to be the best one because the provided information was soon forgotten; moreover, there was the risk of misinterpreting the results, or avoiding being tested because of its "mandatory" character. The Army was also considered; it had been a good target group for epidemiological surveys but here again the information was not seriously considered. The main effort was thus put on couples to be married who had every interest

to know prior to bearing children and represented an immediately accessible group.

Another issue has been that of raising awareness. The mass media have played a significant role in informing people; the ever difficult problem is how to persuade them to donate more viewing time to this effect. Posters and leaflets related to thalassaemia proved equally effective, but these must be continuously made available and renewed. Obstetricians and antenatal clinics came later into play, but now have an important role because they include the carrier identification tests in the routine evaluation of all pregnant women. The Church did not interfere in the programme; after their initial contribution in making available some information leaflets they opted to remain uninvolved.

Overall, the large majority of young people today appear to have adequate information regarding thalassaemia; however, their sensitivity, *i.e.* their fear of bearing an affected child, is lower compared to earlier years, because the dreadful sight of affected patients do not exist anymore. To this effect, more information is necessary and, within this context, the schools acquire a major role; in fact, the topic of haemoglobinopathies has been introduced in biology classes in the secondary schools. However, this is not as easy as it may appear; experience shows that success depends more on the teachers, who need to be adequately educated and enthusiastic to spread the concept to their pupils, than on the printed material.

Currently, an additional problem is that of conveying adequate information to the minorities who have invaded Greece over the recent years, *i.e*., large numbers of migrant Albanians, Pakistanis, Afghans, African and people from the Far East. Obviously, approaching these groups requires educated social workers of the same ethnic background and language, but this step has not been implemented as yet, because other problems have taken priority.

The Greek Ministry of Health has provided ample support in the initial steps of the overall project. A small Unit in Athens, through the help of some written information for doctors, some leaflets distributed through the church offices issuing the prenuptial documents and a lot of voluntary work, rapidly expanded to a network covering the whole country and providing carrier identification to all prospective couples free of charge. Today, the network comprises 15 carrier identification units attached to peripheral hospitals in areas with high frequency of the deleterious genes.

Choice of the appropriate methodology is also of utmost importance; what is critical is the simplicity and low cost of method, and that it includes continuous quality controls and validation of the results. Our Units in Greece offer a complete blood count, as well as haemoglobin electrophoresis or chromatography along with a sickling test. A clearly low mean corpuscular haemoglobin/mean corpuscular volume (MCH/MCV) along with haemoglobin A_2 determination will detect beta-thalassaemia in most cases. Measurement of iron and ferritin excludes iron deficiency. When in doubt, samples are referred to the Central Unit in Athens for further investigation and molecular studies.

The Greek molecular defects are very heterogeneous; most prevalent is the IVS1-nt 110 defect, followed by the CD39 and the IVS1-nt 6 genotypes[14]. Single carriers are notified by a letter giving reassurance that they are not expected to have any problems related to their trait. Whenever both partners have a thalassaemic defect, they are called to receive more information, usually in a personal discussion with a social worker or another educated person.

Carrier identification leads to the option of *(i)* not having children, *(ii)* selecting another partner, or *(iii)* proceeding to prenatal diagnosis. The choice depends on several variable factors, such as the ethical, legal, social and family constraints that prevail in each population at risk as well as the technical feasibility of safely carrying out the procedure in the given environment. Organization of a prenatal diagnosis service is complex in more than just the technical aspect. Prospective mothers need a lot of psychological support to relieve their feelings of guilt about the affected child, to reinforce their decision to proceed when their husband or his family do not concur, to allay the fear for the examination, and to face selective termination of pregnancy when results show homozygosity. Prenatal diagnosis with the potential perspective of selective abortion has been accepted rather easily by most Greek couples at risk; exceptions are rare and relate to religious or personal beliefs. Exclusions in anticipation of a thalassaemia intermedia or HbS disease, which is now manageable with hydroxyurea have not been recorded.

The first cases of prenatal diagnosis in Greece were carried out in 1975. Aris Antsaklis, an obstetrician, trained in the techniques of foetoscopy with Professor

Fairweather in London and the author, a haematologist, had gained some experience on the biochemical part of the procedure with Blanche Alter at Professor's David Nathan Department in Boston, in the USA. In the early cases, the presence of two thalassaemia genes in the foetus was determined by globin biosynthesis of a minute amount of foetal blood obtained through a fetoscope at the 18-19th wk of pregnancy; the aim was to measure the newly formed beta-globin chains, which had to exceed an empirically set level. This was a delicate, if not dangerous, step, because in Greece most foetuses with thalassaemia major are β^+/β^+, and, in consequence, displayed values which might easily overlap with the heterozygous β^+/β^A condition. Be this as it may, errors were rare and the percentage of positive diagnoses remained impressively close to the expected 25 per cent, thus excluding the possibility of overdiagnosis. At this point, one should praise the courage and perseverance of the mothers, who, in spite of the traumatic experience of the procedure, both physically and psychologically in cases of positive diagnosis, came back for prenatal diagnosis of a subsequent pregnancy[15].

The first cases of prenatal diagnosis were reported in 1980[16]; thereafter, the procedure continued being carried out smoothly[17]. Today, prenatal diagnosis is carried out on foetal DNA extracted from chorionic villi cells which are obstetrically obtained at the 8-10th wk of pregnancy. Identification of the noxious genes can be carried out usually following PCR amplification by allele specific hybridization or other molecular techniques. The service is provided solely at the Thalassaemia Unit at Laikon Hospital in Athens at a rate of 500-600 cases annually, which represents the estimated total demand. While having the pregnant women come to Athens for this procedure may appear as a disadvantage, Greece is relatively a small country and the level of safety and quality of the test performed in Athens outweighs the inconvenience. Based on the annual rates, over the past 35 years, approximately 12,000 couples at risk have managed to have a healthy child, while another 4,000 affected foetuses were never brought to life (Thalassemia Center, Athens, Greece, unpublished data). Undoubtedly, the last figure is regrettable, but under present conditions, and until newer methods of treatment, such as gene therapy, or pre-implantation techniques become broadly available, it is probably for the better.

Proof for the above comes through the registration of infants reporting to the major hospital of the country in need for transfusion for thalassaemia major or hospitalisation for sickle cell disease. The number of new cases remains low, 5-10 individuals per year a mean of 15 yearly (two thirds with thalassemia major, another third with HbH and SCD [Voskaridov *et al*, personal communication]. Analysis of the reasons why these cases have been missed shows that some of the parents (in many instances, migrant workers), had not received adequate information, while others simply chose not to consider seriously the given information. Still some other cases relate to non-acceptance of prenatal diagnosis for religious or personal beliefs or errors in the diagnosis of the heterozygous parents or the prenatal testing (Thalassemia Center, Athens, Greece, unpublished data). Of course, efforts to avoid repetition of whatever is avoidable are on the way.

Similar, equally effective programmes are now ongoing in several places of the world[18-21]. What is interesting about the Greek programme is that it started from the beginning as a nationwide government-sponsored and prospectively organised programme which, through the efforts described here, is now giving the anticipated results. Through effective prevention, the impact of new cases of thalassaemia major is reduced thus making it possible to direct the available resources towards the optimisation of treatment of the patients who are with us today.

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